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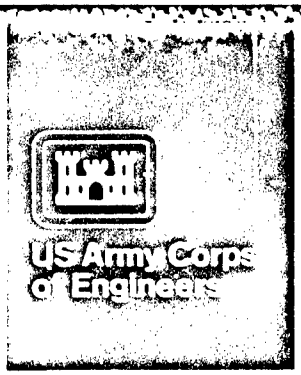

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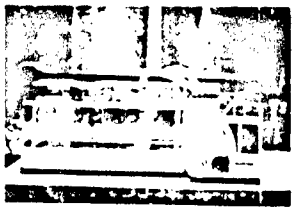

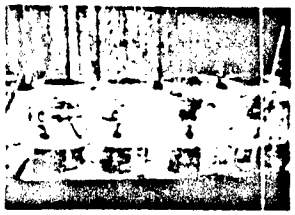

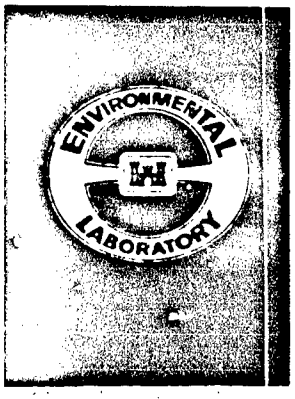

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CULTURE AND TOXICITY TESTS USING LOS ANGELES DISTRICT BIOASSAY ANIMALS, *ACANTHOMYSIS* AND *NEANTHES*

by

Henry E. Tatem, A. Susan Portzer

Environmental Laboratory

DEPARTMENT OF THE ARMY
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LC_{50} values	Polychaete	

20 ABSTRACT (Continue on reverse side if necessary and identify by block number)

This study describes culture techniques and results of toxicant bioassays using the California mysid, *Acanthomysis sculpta*, the polychaete, *Neanthes arenaceodentata*, and the gulf coast mysid, *Mysidopsis bahia*. *Acanthomysis sculpta* was cultured successfully in the laboratory using methods previously developed for *M. bahia*. The *M. bahia* were cultured at 20 ppt salinity and 25°C; *A. sculpta* were cultured at 30 ppt salinity and 15 .

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20. ABSTRACT (Continued).

The mysids were tested with three toxicants: dodecyl sodium sulfate (DSS), also known as sodium laurel sulfate; mercury (Hg); and polychlorinated biphenyls (PCB). The LC_{50} value at 72 hr for DSS showed the California mysid (0.96 ppm) more sensitive to this standard toxicant than the gulf coast mysid (3.80 ppm). Results of the Hg bioassays indicated that the California mysid was more tolerant of Hg than the gulf coast mysid. The Hg LC_{50} at 72 hr for the California mysid was 8.6 ppb compared to 4.4 ppb for the gulf coast mysid. It appears that the California mysid, in comparison to the gulf coast mysid, was more sensitive to DSS, but less sensitive to Hg. Results from PCB (Aroclor 1254) bioassays showed relatively little difference between species (*A. sculpta* - 12.5 ppb; *M. bahia* - 14.2 ppb - LC_{50} at 72 hr).

Neanthes arenaceodentata were held in the laboratory according to established procedures and exposed to only one toxicant, DSS. In comparison to the mysids, the polychaete (8.0 ppm - LC_{50} at 72 hr) was not as sensitive to DSS.

The Section 103 Implementation Manual recommends the gulf coast mysid as an internal standard for dredged material bioassays. Results from this study indicate that both the California mysid and the polychaete are also acceptable bioassay animals, and could be used for dredged material or other toxicity testing.

Preface

This report describes culture techniques and results of reference toxicant bioassay studies using two Los Angeles District bioassay organisms.

The work was conducted during the period of June-August 1983 by the Environmental Laboratory (EL), US Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss., in response to a specific request made by Mr. Russ Belmer of the Los Angeles District. The District needed information on specific test organisms used in their bioassay program. Funding was provided by the US Army Engineer District, Los Angeles.

The experiments were planned and accomplished by Dr. Henry E. Tatem and Ms. A. Susan Portzer with technical assistance provided by Ms. Jerri G. Sims and Mr. Bobby J. Franklin, all of the Ecosystem Research and Simulation Division (ERSD), EL. Editorial review was performed by Ms. Jamie W. Leach of the WES Publications and Graphic Arts Division.

This study was performed under the general supervision of Drs. Richard K. Peddicord and Charles R. Lee, Contaminant Mobility and Regulatory Criteria Group; Mr. Donald L. Robey, Chief, ERSD; and Dr. John Harrison, Chief, EL.

Director of WES was COL Allen F. Grum, USA. Technical Director was Dr. Robert W. Whalin.

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Contents

CULTURE AND TOXICITY TESTS USING LOS ANGELES DISTRICT BIOASSAY ANIMALS, *ACANTHOMYSIS* AND *NEANTHES*

Introduction

Background

1. Personnel of the Los Angeles District contacted the Environmental Laboratory (EL) of the US Army Engineer Waterways Experiment Station in early FY 1983 concerning a need for additional information on organisms used by the District for bioassay testing. Most of the species being used at that time were not common laboratory test animals. They had not been tested with reference toxicants or cultured in the laboratory. It was agreed that the District would obtain and ship the test species to EL, where laboratory cultures would be established for those species with short life cycles. Species supplied by the District would be exposed to reference toxicants such as dodecyl sodium sulfate (DSS), mercury (Hg), and polychlorinated biphenyls (PCB). Lee (1980) has discussed reference toxicants. They can be used to measure the relative sensitivity of test species and/or to compare different batches of test animals. The first animal to be tested was the California mysid, *Acanthomysis sculpta*. Bioassays were also conducted using the polychaete, *Neanthes arenaceodentata*, and the gulf coast mysid, *Mysidopsis bahia*. Work began in June 1983 when the first shipment of animals was received.

Objectives

2. The objectives of this work were to:

   a. Describe procedures for holding and culturing the mysid shrimp *A. sculpta* and the polychaete *Neanthes arenaceodentata*.

   b. Report the results of acute bioassays with the reference toxicants.

   c. Compare results from the California mysid tests with those from similar tests conducted with the gulf coast mysid, *Mysidopsis bahia*.

3. The gulf coast mysid, *M. bahia*, has been used extensively in laboratory toxicity tests (Nimmo and Hamaker 1982). If both mysid species show similar responses, then it can be assumed that the California mysids would be affected by a variety of chemicals at levels similar to those shown to be harmful to *M. bahia*. The Section 103 Implementation Manual (US Environmental

Protection Agency/Corps of Engineers (USEPA/CE) 1977) recommends the gulf coast mysid as an internal standard for dredged material bioassay tests. The California mysid could fulfill this role for Pacific Coast dredged material bioassays if similar responses are established. Literature on culturing mysids (Nimmo, Hamaker, and Sommers 1978; Reitsema and Neff 1980) and polychaetes (Reish 1980a and 1980b) was reviewed and is discussed in relationship to the results of this study.

Acanthomysis sculpta and *Mysidopsis bahia*

Holding and culture

4. California mysids received in June 1983 were collected by Marine Bioassay Laboratories, Watsonville, Calif. Water temperature and salinity at the collection site were approximately 12°C and 32 ppt, respectively. Animals were shipped in natural seawater in plastic bags with oxygen added, methods similar to those used to ship tropical fish.

5. The bags containing the mysids were placed in insulated boxes with cold packs. Shipping time (San Francisco airport to Jackson, Miss.) was approximately 20 hr. The animals arrived in good condition with only one or two dead out of 200 adults and juveniles. Salinity was 32 ppt; however, temperature had risen to 16°C. Many of the adult mysids were carrying eggs. Some of the mysids showed slight signs of stress by repeated contraction of the abdomen. After removal of unwanted additional species, such as small humpback shrimp, the mysids were placed in glass aquaria set in temperature-controlled water baths. During the next 2 days, the water was changed from natural seawater to artificial seawater (salinity 30 ppt). Temperature was held at 15°C. The mysids were fed brine shrimp nauplii. Charcoal aquarium filters were used to aerate the aquaria and filter the water.

6. Since one objective of this study was to culture the California mysids, toxicity tests were not started until stock cultures of the animals (adults and juveniles) were set up to produce young. This was done using techniques developed for the laboratory culture of the gulf coast mysids, *M. bahia* and *Mysidopsis almyra* (Nimmo, Hamaker, and Sommers 1978; Reitsema and Neff 1980).

7. The basic setup, called a mysid generator, consisted of two aquaria: a 20-gal (75 ℓ) aquarium containing the stock culture and a smaller aquarium

containing the juvenile retention chambers (Figure 1). These chambers were petri dishes with a cylinder of nylon screen attached by clear silicone caulk. Both aquaria contained artificial seawater (15°C and 30 ppt) and charcoal filters. Water was pulled from the small aquarium using an overhanging aquarium filter/pump and flowed into the large aquarium. Water from the large aquarium continuously flowed into the small aquarium via a siphon with a large, screened opening. The newly released juvenile mysids were pulled into the retention chambers. The juvenile and adult animals in the stock culture remained in the larger aquarium. A mysid generator was constructed after observing a similar system at the EG&G Bionomics Marine Laboratory in Pensacola, Fla.

8. EG&G supplied the EL with a stock culture of the gulf coast mysid. Both gulf coast and California mysids were cultured using the setup shown in Figure 1 with the only differences being culture temperature and salinity. Gulf coast mysids were held at 25°C while the California mysids were held at 15°C. In our experience, the California mysids will not survive for long at temperatures above 18° to 19°C. Salinity for the California mysids was 30 ppt

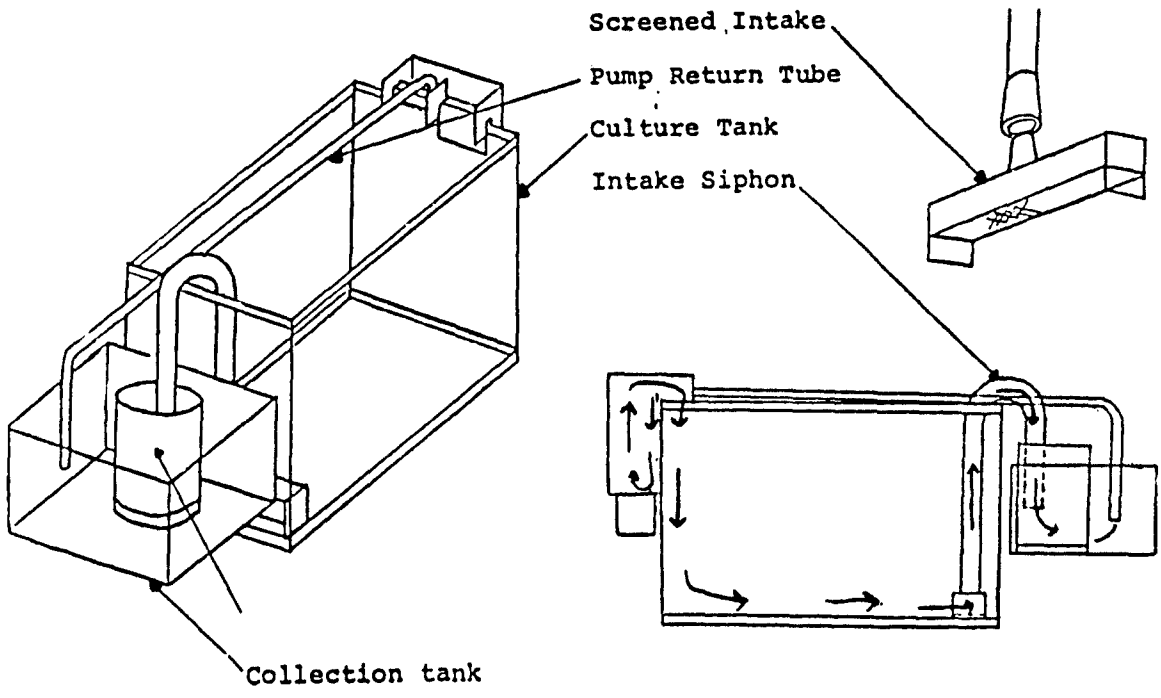

Figure 1. Mysid generator (drawing furnished courtesy of EG&G Bionomics Marine Research Laboratory)

compared with 20 ppt for the gulf coast species.

9. Stock aquaria for both species of mysids contained approximately 100 adults. These produced approximately 50 young every 2 days; however, this was highly variable. Four generator setups were used to ensure a sufficient number of young for testing each week. With the proper attention, laboratory cultures of mysids could be maintained indefinitely; however, it was not unusual for the adults to slowly die over a period of 3 to 5 months in the laboratory.

Toxicity test methods and results

10. Both species of mysids were exposed to the reference toxicants using glass beakers and static exposure conditions. Stock solutions were prepared by adding measured amounts of DSS or Hg to clean artificial seawater; for PCB the stock solutions were prepared by adding Aroclor 1254 to acetone since PCB does not easily dissolve in water. Similar experimental conditions were used for both species so that direct comparisons of the data could be made.

11. Mysid bioassays were conducted using 5 or 10 young (less than 72 hr old) mysids exposed for 72 hr. Four beakers were used at each concentration. Exposures were static with one complete water change and reexposure at 24 hr. The test beakers were not aerated because a small number of mysids in 500 ml of solution did not result in low dissolved oxygen levels. California mysids were tested at 15°C; the gulf coast mysids were tested at 25°C. Exposure concentrations were calculated based on the weight of toxicant added. Animals were counted every 24 hr and fed brine shrimp nauplii. Overall test procedures followed guidelines described in Standard Methods (American Public Health Association (APHA) 1980 and American Society for Testing and Materials 1980).

12. DSS. Results of the initial range-finding bioassay with DSS showed both mysid species affected (100-percent mortality after 72 hr) at 10 ppm DSS. At 1 ppm DSS, survival of the California mysids was 80 percent compared with 100-percent survival for the gulf coast mysids. Both species showed greater than 90-percent control survival.

13. Based on the range-finding results, two additional DSS bioassays were conducted. Both mysid species were exposed to four concentrations of DSS with 4.0 ppm being the highest concentration tested (Tables 1 and 2). Results from both tests for each animal were very similar. California mysids were shown to be more sensitive to the DSS compared with the gulf coast animals.

Control survival at 72 hr for *A. sculpta* ranged from 77.5 to 90.0 percent, compared with 100 percent for *M. bahia* (Tables 1 and 2). These results suggest that the California mysids are more sensitive to laboratory handling during toxicity testing.

14. Data were analyzed using the Litchfield-Wilcoxon technique (Litchfield and Wilcoxon 1949, Weber and Peltier 1980), which allows calculation of the LC_{50} and the 95-percent confidence intervals. An example of a Litchfield-Wilcoxon analysis is given in Appendix A. The 72-hr LC_{50} calculations for the second and third DSS bioassays with the mysids are presented in the following tabulation. These data clearly demonstrate that the California mysid is more sensitive to DSS that the gulf coast mysid. The mean control survival of the

DSS Bioassay	Species	LC_{50} at 72 hr (95% confidence interval)
Second	*A. sculpta*	0.94 ppm (0.77 - 1.15)
	M. bahia	4.50 ppm (3.29 - 6.17)
Third	*A. sculpta*	0.96 ppm (0.77 - 1.20)
	M. bahia	3.80 ppm (2.82 - 5.13)

California mysids for the three tests was 87.5 percent. The guidance in the Implementation Manual (USEPA/CE 1977) that greater than 10-percent control mortality is unacceptable is a difficult goal to achieve. Fifteen- or even twenty-percent control mortality of small, sensitive species such as mysids can be considered acceptable. Therefore, this study indicates that these animals can be used in laboratory toxicity tests even though they are somewhat sensitive to handling.

15. These results can be compared with other DSS toxicity results in the literature. LaRoche, Eisler, and Tarzwell (1970) recommended use of DSS as a standard toxicant for animals being used in bioassay tests. They found a 96-hr LC_{50} of 4.5 ppm DSS for the fish *Fundulus*. Anderson et al. (1974) reported DSS toxicity for the mysid *M. almyra* at 2.0 ppm DSS (24-hr LC_{50}) with higher values for estuarine fish. Tatem, Anderson, and Neff (1976) showed that grass shrimp, *Palaemonetes*, varied in their response to the standard toxicant DSS depending on the time of year. These shrimp revealed LC_{50} values (24 to 96 hr) as high as 100.0 ppm DSS.

16. Thus, the California mysids appear to be more sensitive to the standard toxicant DSS than the gulf coast mysid and some other estuarine

organisms. The California mysids seem to be acceptable bioassay organisms due to their sensitivity to a standard toxicant, their successful laboratory cultivation, and their expected control survival of greater than 85 percent.

17. Mercury. Stock solutions of mercury, $HgCl_2$, were prepared using artificial seawater. Stocks for the California mysids were prepared with 30 ppt seawater while those for the gulf coast mysids were at 20 ppt. As with the DSS, the first step was to conduct an initial range-finding bioassay. Both species were exposed to nominal Hg concentrations of 0.1, 1.0, 10.0, and 100.0 ppb. Both species showed 100-percent mortality at 100.0 ppb. At the 1.0-ppb level, the California mysids showed 70-percent survival, whereas the gulf coast species revealed only 30-percent survival. Control survival, for the range-finding bioassay, was 90 percent for the California mysids and 100 percent for the gulf coast mysids.

18. A final Hg bioassay was conducted by exposing the mysids to Hg at concentrations of 1.0, 5.0, 10.0, and 20 ppb. Both species showed greater than 50-percent mortality at 10.0 ppb Hg. All data from the final Hg bioassay are presented in Table 3. Results of the Litchfield-Wilcoxon analysis are shown below.

Species	LC_{50} at 72 hr (95% confidence intervals)
A. sculpta	8.6 ppb (6.23 - 11.87)
M. bahia	4.4 ppb (2.80 - 6.91)

19. Results of the Hg bioassays indicate that the California mysid seems to be more tolerant of Hg compared with the gulf coast mysid. The gulf coast mysids have been exposed to Hg by others (Gentile et al. 1983). They found Hg to be acutely toxic to juvenile mysids (96-hr LC_{50} of 3.5 ppb) at levels close to those reported here. Chronic toxicity was observed at levels of 1.8 ppb Hg. The results from the range-finding Hg bioassay and the LC_{50}'s calculated by using the Litchfield-Wilcoxon technique indicate that the California mysid was slightly more resistant.

20. PCB. The PCB stock solutions were prepared by adding a known amount of Aroclor 1254 to acetone. The Aroclor was 100-percent pure (Lot No. D022) and was received from the USEPA, Research Triangle Park, N. C. There were five mysids per beaker, four replicates per PCB concentration, and four different concentrations tested. Tests were conducted for 72 hr with one complete water change/reexposure at 24 hr. The PCB was a clear, oily liquid.

A glass rod was used to transfer PCB from the shipping vial to a preweighed square of aluminum foil. The aluminum foil, with the PCB, was placed in a known volume of acetone to produce the acetone-PCB stock. The PCB dissolved and known volumes of acetone stock were added to exposure beakers containing the mysids and artificial seawater.

21. Two PCB bioassays were completed using nominal (calculated) PCB concentrations of 0.2, 0.5, 1.0, 10.0, and 50.0 ppb PCB (Tables 4 and 5). Control survival for both species was 90 to 100 percent. The acetone controls were beakers containing mysids exposed to acetone without PCB (APHA 1980). For the second PCB test both species showed 100-percent control survival and 95-percent acetone control survival. Results of the second bioassay showed that mysids (both species) exposed to 1.0 and 10.0 ppb PCB for 72 hr exhibited 85- to 100-percent survival while those exposed to 50 ppb exhibited 0-percent survival. The estimated LC_{50}'s are shown below. They could not be calculated with confidence limits since no concentration showed partial survival less than 50 percent.

PCB Bioassay	Species	Estimated LC_{50} at 72 hr
First	*A. sculpta*	14.5 ppb
	M. bahia	12.5 ppb
Second	*A. sculpta*	22.5 ppb
	M. bahia	14.2 ppb

22. The gulf coast mysid has been used in bioassay tests with at least 12 substances (Nimmo and Hamaker 1982); however, PCB was not one of the substances. Nebeker and Puglisi (1974) have reported static LC_{50} (3-week) data for *Daphnia* exposed to PCB 1254 as 31.0 ppb. Our values, at 72 hr, for both species of mysids were below 31.0 ppb, which indicates that they appear to be more sensitive than *Daphnia*, a recognized, sensitive freshwater crustacean. The second test should be given the most emphasis because of the excellent control survival. Results of these tests show relatively little difference between the two mysid species with respect to their response to PCB.

Neanthes arenaceodentata

Holding and culture

23. Approximately 400 juvenile *Neanthes* polychaetes were ordered from D. Reish (Biology Department, California State at Long Beach, Calif.) in July

1983. Animals arrived by air in good condition and were placed in small glass aquaria or polyethylene trays containing clean sand. Using artificial seawater at a salinity of 35 ppt and temperatures of 21° to 23°C, the polychaetes were held under static conditions. These animals were fed powdered alfalfa, and the water in the holding containers was replaced weekly. Procedures for holding polychaetes followed Reish (1980a), while testing procedures were from Reish (1980b). The only problem experienced occurred when the laboratory temperature reached approximately 30°C due to failure of the cooling units; polychaetes exposed to this temperature did not survive. These animals are easy to work with in the laboratory and testing procedures are standardized.

Toxicity test methods and results

24. *Neanthes* were exposed to DSS but not to Hg or PCB. Three bioassays were conducted. Ten animals were used, each exposed in individual containers to the DSS. Initial tests showed that *Neanthes* exposed to 10.0 ppm DSS did not survive while those exposed to 5.0 to 7.5 ppm showed 90-percent survival or higher. Data from the final test are shown in the following tabulation:

	Percent Survival		
DDS, ppm	24 hr	48 hr	72 hr
Control	100	100	100
5.0	100	100	100
8.0	100	70	50
10.0	100	30	20
12.0	100	0	0
15.0	10	0	0

Data from the final test were used to calculate the 72-hr LC_{50} (8.0 ppm) and 95-percent confidence intervals (7.14 - 8.96).

25. *Neanthes* have been widely used for laboratory toxicity tests (Reish 1980a); however, most of this work has been conducted using heavy metals and petroleum hydrocarbons. No data were found concerning the acute or chronic toxicity of PCB to these animals (USEPA 1976). The 96-hr LC_{50} for *N. arenaceodentata* exposed to Hg has been reported as 0.02 ppm Hg (Reish et al. 1976). This value is the same as 20 ppb Hg, which is approximately twice the level of Hg shown to be toxic at 72 hr to the California mysid in this study. Comparison of the DSS results for the polychaete (72-hr LC_{50} of 8.0 ppm) and the California mysid (72-hr LC_{50} of 1.0 ppm) also indicates that the

polychaete is not as sensitive to this standard toxicant as the mysid. Data from the literature indicate that, in general, mysids are more sensitive to toxicants compared with the polychaete *Neanthes*.

Summary and Conclusions

26. The California mysid, *A. sculpta*, was collected from the field and shipped in good condition. The animals were successfully held and cultured in the laboratory using artificial seawater and techniques developed for a gulf coast mysid species, *M. bahia*. A second Los Angeles District bioassay animal, the polychaete *N. arenaceodentata*, was received and maintained in the laboratory following established procedures. The mysid species were exposed to three reference toxicants; polychaetes were exposed to the surfactant DSS, but not to Hg or PCB. Mean LC_{50} values, at 72 hr, obtained are shown in the following summary tabulation:

	Mean LC_{50} Values, 72 hr		
Species	DSS, ppm	Hg, ppb	PCB, ppb
A. sculpta	0.95	8.6	18.5
M. bahia	4.15	4.4	13.4
N. arenacecdentata	8.00	—	—

These results indicate that both mysid species are sensitive to the reference toxicants. The California species appears to be more sensitive to the DSS and less sensitive to the Hg compared with the gulf coast mysid. These results plus the literature reviewed indicate that the California mysid is a sensitive bioassay animal. Survival of controls was acceptable. The response of the California mysid to a variety of contaminants, based on these data, could be expected to be similar to the gulf coast mysid. The polychaete species was easily held in the laboratory and tested following standard procedures. It was not as sensitive to the DSS as the mysids but was shown to be affected at levels below 10 ppm DSS. This concentration is below the LC_{50} values reported for DSS toxicity to some other estuarine organisms. Both the mysid and the polychaete appear to be suitable bioassay animals and useful for dredged material toxicity testing. The mysid could be used as an internal standard for Pacific coast bioassay tests.

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Table 1

Survival of Mysids Exposed to DSS in Second Bioassay*

DSS ppm	*A. sculpta* Survivors 24 hr	48 hr	72 hr	Percent Survival 72 hr	*M. bahia* Survivors 24 hr	48 hr	72 hr	Percent Survival 72 hr
Control	10	9	8	90	10	10	10	100
	10	10	10		10	10	10	
	10	10	9		10	10	10	
	10	9	9		10	10	10	
0.5	10	9	8	82.5	10	10	10	90
	10	8	9		10	9	9	
	9	9	7		10	9	8	
	9	9	9		10	10	9	
1.0	10	8	8	52.5	10	10	10	95
	9	7	5		10	9	9	
	10	6	3		10	10	9	
	10	7	5		10	10	10	
2.0	9	1	1	5	10	10	9	92.5
	8	4	1		10	10	10	
	10	3	0		10	10	10	
	7	1	0		10	8	8	
4.0	4	0	0	0	9	9	9	52.5
	4	0	0		6	4	4	
	4	0	0		8	7	4	
	1	0	0		6	5	4	

* There were 10 animals in each of four replicate containers initially.

Table 2

Survival of Mysids Exposed to DSS in Third Bioassay*

DSS ppm	*A. sculpta* Survivors 24 hr	48 hr	72 hr	Percent Survival 72 hr	*M. bahia* Survivors 24 hr	48 hr	72 hr	Percent Survival 72 hr
Control	9	8	8	77.5	10	10	10	100
	9	9	8		10	10	10	
	9	9	9		10	10	10	
	8	7	6		10	10	10	
0.5	10	9	8	72.5	10	10	9	92.5
	9	9	8		10	10	9	
	10	8	4		10	10	10	
	10	10	9		9	9	9	
1.0	8	8	5	52.5	10	10	10	97.5
	9	8	6		10	10	10	
	10	9	5		10	10	10	
	9	8	5		10	9	9	
2.0	10	2	1	2.5	9	9	9	87.5
	10	0	0		9	7	6	
	7	1	0		10	10	10	
	8	2	0		10	10	10	
4.0	0	0	0	0	9	5	4	45
	0	0	0		9	5	5	
	2	0	0		10	8	5	
	0	0	0		8	5	4	

* There were 10 animals in each of four replicate containers initially.

Table 3

Survival of Mysids Exposed to Hg*

DSS ppm	*A. sculpta* Survivors 24 hr	48 hr	72 hr	Percent Survival 72 hr	*M. bahia* Survivors 24 hr	48 hr	72 hr	Percent Survival 72 hr
Control	5	5	5	80	5	5	5	90
	5	5	4		5	5	5	
	5	5	4		4	4	4	
	5	3	3		5	5	4	
1.0	5	3	3	90	4	4	4	85
	5	5	5		4	4	3	
	5	5	5		5	5	5	
	5	5	5		5	5	5	
5.0	5	5	5	95	5	3	2	45
	5	5	5		5	2	2	
	4	4	4		5	2	2	
	5	5	5		4	3	3	
10.0	5	3	1	25	5	1	0	30
	5	3	2		5	4	2	
	5	3	2		5	3	3	
	5	2	0		5	1	1	
20.0	5	3	0	5	2	0	0	0
	3	2	1		5	1	0	
	3	1	0		4	1	0	
	1	1	0		4	0	0	

* There were five animals in each of four replicate containers initially.

Table 4

Survival of Mysids Exposed to Aroclor 1254 in First Bioassay*

DSS ppm	*A. sculpta* Survivors 24 hr	48 hr	72 hr	Percent Survival 72 hr	*M. bahia* Survivors 24 hr	48 hr	72 hr	Percent Survival 72 hr
Control	5	4	4	90	5	5	4	90
	4	4	4		5	5	5	
	5	5	5		5	4	4	
	5	5	5		5	5	5	
Acetone control	5	5	5	80	4	4	4	75
	5	4	4		5	5	5	
	3	2	2		2	2	2	
	5	5	5		5	4	4	
0.2	5	5	5	100	5	5	5	90
	5	5	5		5	5	5	
	5	5	5		5	4	3	
	5	5	5		5	5	5	
0.5	5	5	5	100	5	5	5	90
	5	5	5		5	5	4	
	5	5	5		5	5	5	
	5	5	5		5	4	4	
1.0	5	5	5	95	5	5	5	85
	5	5	5		5	4	4	
	5	5	5		5	5	4	
	5	5	4		5	5	4	
10.0	5	3	3	75	4	4	2	55
	5	5	5		5	3	2	
	5	4	4		4	4	2	
	5	3	3		5	5	5	

* There were five animals in each of four replicate containers initially.

Table 5

Survival of Mysids Exposed to Aroclor 1254 in Second Bioassay*

DSS ppm	*A. sculpta* Survivors 24 hr	48 hr	72 hr	Percent Survival 72 hr	*M. bahia* Survivors 24 hr	48 hr	72 hr	Percent Survival 72 hr
Control	5	5	5	100	5	5	5	100
	5	5	5		5	5	5	
	5	5	5		5	5	5	
	5	5	5		5	5	5	
Acetone control	5	5	5	95	5	5	5	95
	5	4	4		4	4	4	
	5	5	5		5	5	5	
	5	5	5		5	5	5	
0.2	5	5	5	95	5	5	5	100
	5	5	5		5	5	5	
	5	5	5		5	5	5	
	4	4	4		5	5	5	
1.0	5	5	5	95	5	5	5	100
	5	5	5		5	5	5	
	4	4	4		5	5	5	
	5	5	5		5	5	5	
10.0	5	5	5	100	5	4	4	85
	5	5	5		3	3	3	
	5	5	5		5	5	5	
	5	5	5		5	5	5	
50.0	3	1	0	0	1	0	0	0
	2	0	0		0	0	0	
	2	0	0		1	0	0	
	0	0	0		2	0	0	

* There were five animals in each of four replicate containers initially.

Appendix A: Litchfield-Wilcoxon Example*

1. To calculate LC_{50} and 95-percent confidence intervals from bioassay data using this technique, it is necessary to have the Litchfield-Wilcoxon paper (Litchfield and Wilcoxon 1949). It contains nomographs for obtaining chi square and making other calculations. The first step is to construct a table and place the data points on probability-log paper (Figure A1).

2. The next step is to calculate the chi square from Nomograph 1. Then determine if the line is a good fit by comparing the calculated chi square with the chi square from Table 2. If the line is a good fit, the LC_{16}, LC_{50}, and LC_{84} can be determined from Figure A1.

DSS, ppm	#React/ #Exposed	% Response	Expected % Response (Figure A1)	Observed Minus Expected	Contributions to Chi Square (Nomo. 1)
0.4	4/40	10	outlier	-	-
1.0	2/40	5	2	3.0	0.045
2.0	3/40	7.5	13	5.5	0.027
4.0	19/40	47.5	44	3.5	0.0035
					0.0755

40 (0.0755) = 3.02 (chi square)

Degrees of freedom = 1

Chi square from Table 2 in Litchfield and Wilcoxon (1949) = 3.84

Calculated chi square less than 3.84 - line is good fit

LC_{16} - 2.2 ppm
LC_{50} - 4.5 ppm
LC_{84} - 9.5 ppm
} from Figure A1

$$\text{Slope function} = \frac{9.5/4.5 + 4.5/2.2}{2} = 2.08$$

$$\sqrt{40} = 6.325$$

$$f \quad LC_{50} = 2.08 \frac{2.77}{6.325} = 1.37 \text{ (Litchfield and Wilcoxon 1949 - Nomograph 2)}$$

95% confidence limits = 1.37 (4.5 ppm) = 6.17 ppm

= 4.5 ppm/1.37 = 3.29 ppm

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* Data from Table 1, *Mysidopsis bahia*.

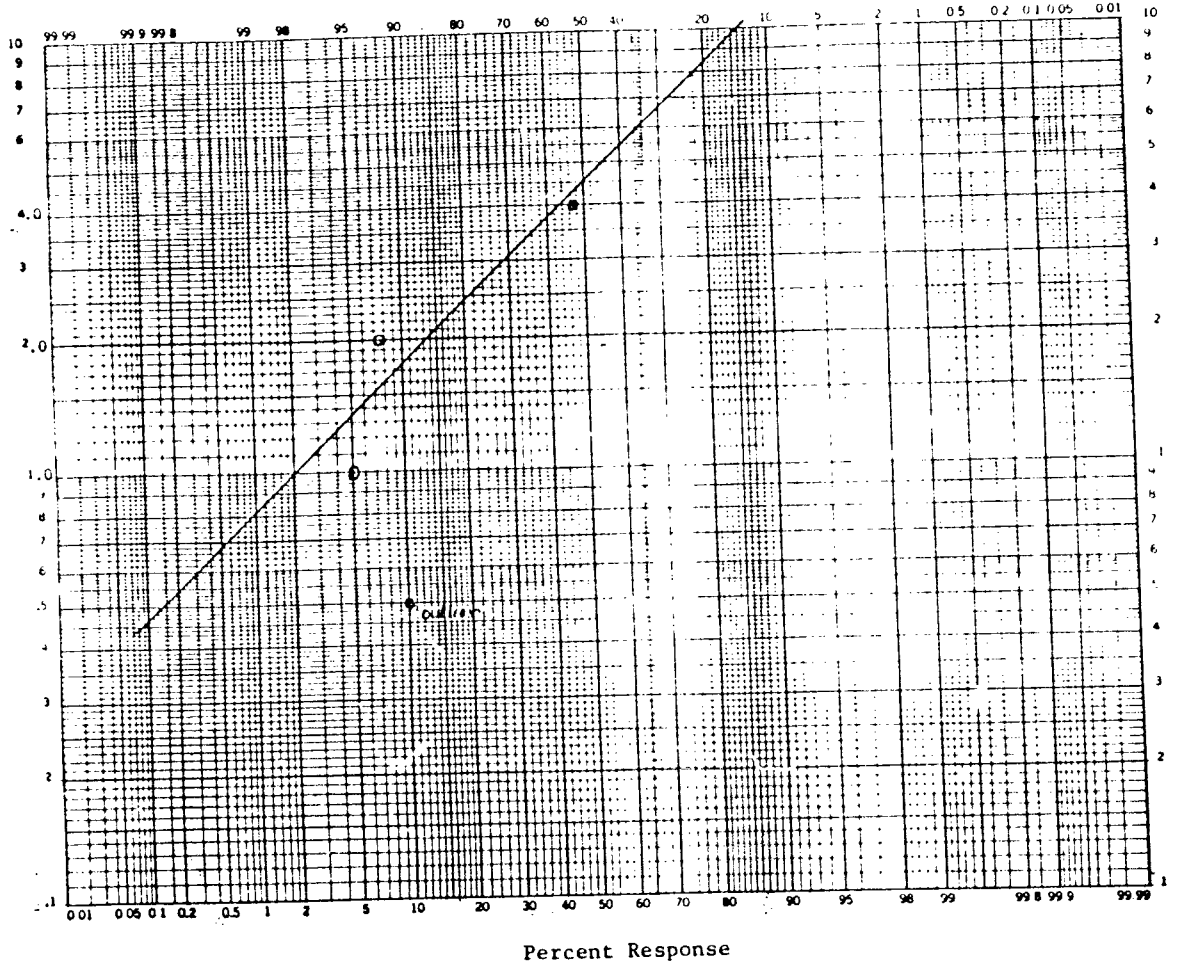

Figure A1. Bioassay data (from Table 1, *M. bahia*) on probability-log paper for use in Litchfield-Wilcoxon analysis